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Heat disinfestation of decay fungi found in post-mountain pine beetle wood

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FPInnovations – Forintek Division

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Abstract

In early stages of mountain pine beetle attack, blue-stain fungi associated with the beetle are the predominant microorganisms in the trees. As post-beetle standing trees age, a succession of other fungi occurs, including both sap- and heart-rot fungi, which may flourish in red- and grey- attack stages. In this study, 22 isolates from later stages of beetle-attacked wood were assembled, representing eight fungus species, to test the effectiveness of heat treatment to eradicate fungi present in wood, (including 56° C for 30 min (56/30) as adopted by ISPM-15 international standard) and whether slow drying/desiccation increased heat tolerance of selected isolates. Nine isolates survived 56/30, but all were killed by 61° C for 30 min. Results indicated that some fungi may increase their heat tolerance slightly if they have been slowly drying/desiccating prior to treatment. Considering the insulating capacity of wood and that industrial ovens achieve higher temperatures near the wood's edge and longer core heating times in meeting the ISPM-15 target of 56/30 in the wood's core, schedules that achieve 56/30 in the core will be sufficient to eradicate most undesirable fungi associated with mountain pine beetle. Also, naturally occurring, aggressive mould fungi will further reduce the chance of surviving fungi to escape from treated wood once it reaches the marketplace. It is hoped that these findings will improve the understanding of perceived versus real threats by fungal organisms associated with beetle-affected wood that may survive 56/30 under lab conditions, and prevent any unsubstantiated restrictive trade practices.

Keywords: MPB, mountain pine beetle, fungi, decay fungi, sap rot, heart rot, basidiomycetes, heat treatment, phytosanitary, temperature tolerance, 56/30

Résumé

Aux premiers stades d'une infestation de dendroctone du pin ponderosa (DPP), les champignons de bleuissement associés au dendroctone sont les principaux microorganismes détectés sur les arbres. À mesure que les arbres infestés par le DPP vieillissent, ils deviennent la cible d'une succession d'autres champignons, notamment de champignons de pourriture de l'aubier et de pourriture du cœur, appelés à devenir prédominants aux stades rouge et gris. Dans cette étude, 22 isolats du bois aux derniers stades de développement d'une infestation de DPP ont été assemblés pour tester l'efficacité d'un traitement thermique visant à éradiquer les champignons présents dans le bois (notamment à 56 C pendant 30 min (56/30), conformément à la norme internationale NIMP-15) et pour étudier la possibilité qu'un procédé de séchage lent ou de dessiccation puisse accroître la tolérance thermique des isolats selectionnés. Neuf isolats ont survécu au traitement 56/30 mais tous ont été anéantis lorsqu'exposés à 61° C pendant 30 min° C. Ces résultats ont indiqué que certains champignons pourraient développer une petite tolérance à la chaleur lorsqu'on les soumet à un séchage lent ou à une dessiccation préalable au traitement thermique. En conséquence des propriétés isolantes du bois ° Cet que les fours industriels entraînaient parfois des températures plus élevées dans les couches extérieures du bois et un temps de chauffage accru au centre en visant la norme NIMP-15 dc 56/30, les programmes atteignant 56/30 au centre seront suffisants pour éradiquer la majorité des champignons indésirables associés au DPP. Par ailleurs, la présence naturelle de champignons-moisissures agressives réduira encore davantage les chances des champignons survivants de s'échapper du bois traité une fois mis sur le marché. On espère que ces résultats permettront d'améliorer la distinction entre menaces réelles et menaces supposées au regard des organismes fongiques associés au bois affecté par le DPP qui pourraient survivre à un traitement 56/30 dans des conditions de laboratoire, en vue de prévenir toute restriction inconsidérée des pratiques commerciales.

Mots-clés : DPP, dendroctone du pin ponderosa, champignon, champignons décomposeurs, pourriture de l'aubier, pourriture du cœur, basidiomycètes, traitement thermique, phytosanitaire, résistance thermique, 56/30

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1 Introduction

The international spread of pest organisms is recognized as a serious issue by the plant health regulatory agencies of governments, the forest industry, and the general public. Questions about such pests carried by wood products arise in the market and are more likely to be asked when wood is known to originate from dead trees. Currently, most wood-inhabiting fungi, including those associated with post-mountain pine beetle (MPB) wood, are not listed as phytosanitary pests of concern, but some pathogenic fungal species are monitored by a few countries. It is estimated that up to 900 million cubic meters of lodgepole pine in BC will be killed by 2010, and the portion for export has to meet international phytosanitary regulations. The most commonly applied regulation is the 56° C for 30 min (56/30) heat treatment criteria, permitting dried lumber to be stamped HT or issued a HT certificate for green lumber. The understanding is that heat-treated wood poses much less of a phytosanitary risk than non-processed wood, although 56/30 does not kill all organisms carried by wood.

Studies that developed the 56/30 schedule during trade negotiations between North America and the European Union proved with 0.99994 reliability (probability of a successful treatment) that 56/30 kills the pinewood nematode (*Bursaphelenchus xylophilis*), and recognized that this schedule would also kill their insect vectors (Smith 1991). The experimental method used resembled the one in this current test, but used nematode-infested wood. Even at 52° C, no nematodes survived any of 60 nematode-infested and tested replicate pieces. However, statistically this gives only 0.95 reliability. As the trading partner (European Union) requested a kill temperature that will assure higher statistical reliability (0.99994), the required kill-temperature was extrapolated to 56° C following Gompertz model mortality curve developed during the study. Given that it is reasonable to assume that the 56/30 would be effective against other nematodes and insects, this temperature–time schedule has been since adopted as an international phytosanitary measure by the International Plant Protection Convention (IPPC) that addresses the need to treat certain softwood packaging materials under International Standard of Phytosanitary Measures (ISPM) No15 (Secretariat IPPC 2006). It was hoped that this HT treatment would significantly reduce live pest transfer and let trade continue. Initially, ISPM No15 focused on insect and nematode pests; however, questions have since been raised on fungal pathogens and the efficacy of 56/30 to kill them.

Literature on heat sterilization of fungi indicates some wood-inhabiting fungi survive heating to 56° C or higher (Chidester 1937; Newbill and Morrell 1991; Allen 2001). The significance and importance of these surviving fungi in the context of real-life situations, where pathways to infect exotic hosts need to be established, is largely unknown.

This study is related to the Canadian Forest Service (CFS) Mountain Pine Beetle Initiative (MPBI) strategic initiative 1.B.1: "Development and delivery of market support information on the aesthetic and performance properties of post-beetle wood". It follows the project "Heat Disinfestation of Mountain Pine Beetle-Affected Wood," funded by the MPBI, where we studied the efficacy of several time/temperature combinations, including 56° C for 30 min, to eradicate fungal species found in the early stage of beetle attack (Uzunovic and Khadempour 2007). In the initial stages, blue-stain fungal associates of the beetle, including *Ophiostoma clavigerum*, *O. montium*, *Leptographium longiclavatum*, *L. terebrantis* and *Ambrosiella* sp., are the most common. Two basidiomycetes, *Trichaptum abietinum* and *Phellinus chrysoloma*, that have been reported in early stages of colonization (Kim et al. 2005) were also included.

Fungal diversity usually increases as post-beetle wood ages, depending on many factors but most importantly whether the moisture conditions of standing dead trees allows subsequent colonization by other insects and fungi. Saprophytic fungi, including sap-rot and heart-rot fungi, have been observed in later stages of attacked trees and in lumber (Kim et al. 2005; Lewis and Hartley 2006).

With the Canadian Forest Service, Pacific Forestry Centre, FPInnovations – Forintek Division developed a method for testing fungal survival after phytosanitary heat treatment. The protocol has been posted on

the website of the International Forestry Quarantine Research Group (IFQRG) (Uzunovic et al. 2006) after being reviewed by international experts. This method was used in our previous test (Uzunovic and Khadempour 2007) and slightly modified for current tests. It has also been used in other countries such as New Zealand (Mike Ormsby, personal communication) where they tested fungi typically found in wood products or fungi of biosecurity concern to New Zealand.

This research focuses on studying the heat tolerance of sap-rot and heart-rot fungi found in beetle-killed wood. Specifically, we evaluate the 56/30 schedule and other temperature/time combinations. We also investigated whether slow air-drying increased the heat tolerance of the fungi. The generated data are expected to fill an important science gap on the fungi found in beetle-affected trees and enable international discussions on post-beetle lumber to be soundly based on scientific facts.

2 Material and Methods

2.1 Decay Fungi Selection

We selected test isolates primarily based on a literature review that included recently published work on fungi associated with the mountain pine beetle as well as the Centraalbureau voor Schimmelcultures database (www.cbs.knaw.nl) and discussions with several key researchers including Colette Breuil and Sepideh Alamouti of the Department of Wood Science, University of British Columbia (UBC). Some fungi came from work done by Kathy Lewis, University of Northern British Columbia (UNBC), who collected naturally infested materials from two British Columbia locations (Lewis 2008). After the review, chosen putatively identified isolates were obtained from UBC's Department of Wood Science culture collection. We selected eight decay species, each represented by three isolates (where available) from different British Columbia geographic origins. All isolates were basidiomycetes taken from later stages of beetle-killed wood representing both sap-rot and heart-rot fungi as well as those that do or do not produce chlamydospores. The following fungi were tested: *Peniophora* sp., *Entomocorticium* sp., *Fomitopsis pinicola*, *Phlebia tremellosa*, *Stereum sanguinolentum*, *Amylostereum chailletii*, *Phellinus pini*, and *Sistotrema brinkmannii* (Table 1). Throughout the test we used a replication of six for each isolate for each tested parameter.

Table 1. List of test fungal isolates, type of decay they cause and test treatment

Species	Code	Associated decay	Treatment
Peniophora sp.	A	Heart rot	HT
	B	Sap rot	
	C	Heart rot	
Entomocorticium sp.	D	Unknown decay capabilities	HT
	E		
*Fomitopsis pinicola**	F	Heart rot	HT and DHT
	G		
	H		
*Phlebia tremellosa**	I	Sap rot	HT and DHT
	J		
	K		
Stereum sanguinolentum	L	Heart rot	HT and DHT
	M		
	N		
Amylostereum chailletii	P	Sap rot	HT and DHT
	Q		
	R		
Phellinus pini	S	Heart rot	HT
	T		
	U		
Sistotrema brinkmannii	V	Wood inhabiting but non-wood rotting basidiomycete	HT
	W		
	X		
	Y		

HT – immediate heat treatment; DHT – drying then heat treatment: 90 days of air-drying prior to heat treatment

*F. pinicola and P. tremellosa were known to produce chlamydospores (Stalpers 1978). Sistotrema brinkmannii sensu lato is also reported to produce chlamydospore-like structures (Hallenberg 1984)

2.2 Inoculation and Incubation of Samples

The inoculation method was based on the previous heat treatment study with some changes to accommodate specific needs of decay fungi and to colonize test samples well. Preliminary studies determined whether re-wetted stained sapwood or stain-free fresh green sapwood was better for growing sap-rot fungi. The fungi grew better on clean green sapwood so this substrate was used for sap-rot fungi.

Clean healthy green lodgepole pine logs were obtained, cut into test sample sizes of 30 × 10 × 5 mm, and sent to Iotron Industries Canada Inc. (Port Coquitlam, BC) for sterilization by irradiation at 25kGy. After sterilizing, while keeping the green condition, samples were inoculated with individual test isolates as described in Uzunovic and Khadempour (2007), and left to incubate at 20° C–23° C (lab room temperature) until sufficient fungal colonization was achieved (6–10 weeks).

Since many heart-rot fungi were tested, we grew them on their natural heartwood substrate. Preliminary testing with *Fomitopsis pinicola* and *Stereum sanguinolentum* on heartwood was successful, but they grew faster on sapwood, and the former fungus did not colonize sufficiently for testing until after 8–10

weeks of growth (in contrast to 6–8 weeks). Extra moisture was added to heartwood samples to encourage and speed the fungal growth.

Since *Peniophora* sp. isolates were identified only to genus, it was not known whether they were heart-rot or sap-rot fungi. Tests indicated that isolates A and C could grow on heartwood, so they were categorized as heart-rot fungi. Isolate B was not able to grow on heartwood, so it was categorized as a sap-rot fungus.

After inoculating samples in the pre-sterilized breathable-patch bags (Western Biologicals Ltd. Cat. No. 3TL) containing saturated sterilized vermiculite; the bags were maintained at room temperature. The bags have an air-permeable patch and are designed for mushroom spawn production. Moisture in the bags was monitored weekly and sterile water added aseptically as required. Sacrificial wood pieces were examined to determine whether fungal growth was sufficient for starting treatment and to monitor the development of chlamydospores, heat-resistant structures. Incubation lasted 6–10 weeks depending on the test fungus.

2.3 Desiccation/Drying prior to Heat Treatment (DHT)

Four species (11 isolates) were selected for further experiments where they were exposed to slow drying (DHT) for 90 days prior to heat treatment. Among these, *Fomitopsis pinicola* and *Phlebia tremellosa* were chosen as they produce chlamydospores. Test fungi were air-dried to stress them and help develop resting spores, thus potentially increasing the fungal heat tolerance and allowing for testing of the most resistant stage of a fungus. The two species randomly chosen for air-drying were *Stereum sanguinolentum* and *Amylostereum chailletii*. For these four species, we placed the required amount of inoculated wood samples in an environment chamber at 20° C and 79% relative humidity, targeting an equilibrium of 15% moisture content. After 90 days of drying, samples were heat-treated following the same procedure and replication as for direct heat treatment. We used the same method as in Uzunovic and Khadempour (2007), where six replicates for each time-temperature treatment and three for the control were used.

2.4 Naturally Infested Samples

Heat treatment tests were also performed on both sapwood and heartwood of naturally infested post-beetle lodgepole pine. We obtained logs throughout British Columbia by asking industry members to send us logs cut from trees in red-grey stage that were fully blue-stained and had signs of decay. We also wanted logs from trees from wetter sites, as these trees were more likely to be colonized by decay species. We did not want logs that had a single column of internal decay (e.g., butt rot caused by a single decay species) or logs that had dried out. Upon arrival, logs were kept half-immersed in water for several days to increase their moisture content, then incubated at room temperature for several days and sampled to test for the presence of fungi. We chose the log from which the most diverse mycota was isolated during the preliminary sampling. Test samples (30 × 10 × 5 mm) were cut from this log and one third of the samples were allowed to desiccate in a conditioning chamber prior to heat treatment. Ten undried replicates were allocated for each time-temperature combination from both the heartwood and the sapwood, as well as 10 dried replicates for each time-temperature treatment. The moisture content of the undried heartwood and sapwood was measured on a dry weight basis 58.4% and 73.0%, respectively.

2.5 Heat Treatment Process

Test wood samples were vacuum-sealed with six replicates in each plastic bag, submerged in water at the test temperature for the target time, then cooled in a 25° C water bath ° Cfor 2 min. Samples were aseptically removed from the bags and placed on 1% MEA plates. (*Note: Preliminary tests showed no difference in recovery of fungi on 1% MEA than on other selective media so 1% MEA was used throughout the tests*).

The testing protocol specified in Uzunovic and Khadempour (2007) was changed: smaller vacuum bags and custom-made stainless steel racks were used to run all the samples treated for different times at a specific temperature simultaneously. Monitoring the samples' core temperature showed the modifications

did not affect the heating time. (The concern was that the steel rack might cause an unexpected water temperature drop and slow the heating of test samples). As in Uzunovic and Khadempour (2007), four minutes continued to be the initial heating time before we started to measure exposure time.

Table 2. Time /temperature matrix used for each fungus and for controls

Time (minutes)	Temperature° C							
	25	41	46	51	56	61	66	71
<1	N=3	N=6	N=6	N=6	N=6	N=6	N=6	N=6
30	N=3	N=6	N=6	N=6	N=6	N=6	N=6	N=6
60	N=3	N=6	N=6	N=6	N=6	N=6	N=6	N=6
120	N=3	N=6	N=6	N=6	N=6	N=6	N=6	N=6

Following discussions with the International Forestry Quarantine Research Group, the treatment for 10 minutes in the original plan was considered redundant at this stage and therefore cancelled.

2.6 Assessment of Survival and Recovery Procedure

Immediately following the heat treatment, test samples were placed aseptically on 1% MEA Petri-plates and fungal outgrowth was monitored to note if test fungi survived the treatment. Plated samples were assessed after 7 and 21 days for growth and any unusual features of growth were noted. Plates without any growth (0) were checked once more after an additional 30 days of incubation following the 21-day assessment. Unusual samples were checked under a microscope or sub-cultured for later assessment of growth rate and morphology. Subcultures were macroscopically compared to the original culture to determine whether the test time/temperature affected the appearance of each test fungus.

For naturally infested wood, each treated wood piece was split in two halves longitudinally with a sterile razor blade. One part was plated on 1% MEA plate amended with 0.01% chloramphenicol (to suppress bacterial growth) and the other part was plated on media amended with 0.01% chloramphenicol and 0.002% benomyl. Benomyl is an important ingredient in basidiomycete-selective media because it inhibits the growth of antagonistic micro-organisms. The survival assessment was done by enumerating types of fungi occurring on both media observed under a dissecting microscope. We specifically concentrated on whitish non-sporulating mycelia of a particular shape that occurred on benomyl-amended media as these were most likely to be decay fungi. Some of these were observed under a compound microscope to confirm the presence of clamp connections, which is a typical morphological feature found in basidiomycetes.

3 Results

3.1 Heat Treatment – Inoculated Test Wood with Test Isolates – No Air-drying

Among eight tested fungal species, represented by 22 isolates in total, three species survived 56/30 for a total of nine isolates (Table A1). These included *Peniophora* sp, *Phlebia tremellosa* and *Phellinus pini*. For *Peniophora* sp and *P. tremellosa*, all three isolates and all replicates survived. For *P. tremellosa* two replicates of one isolate did not survive 56/30. All isolates and replicates of *Peniophora* sp and *P. pini* survived 56° C for 60 minutes and some even for 120 minutes, while few replicates of two isolates of *P. tremellosa* survived 56° C for 60 minutes. All isolates representing all eight species were killed at the next higher tested temperature (61° C) for 30 minutes. No isolates survived any temperature/time combinations beyond 61° C /30 minutes.

3.2 Heat Treatment after Air-drying for 90 Days

Results for isolates first subjected to slow air-drying for 90 days then heat treated (DHT) showed a difference in fungal survival when compared with results of direct heat treatment following incubation (Table A2). Air dying (DHT) was done for four fungal species *Fomitopsis pinicola, Phlebia tremellosa, Stereum sanguinolentum* and *Amylostereum chailletii*, all represented in total by 11 isolates. There was clear evidence for all four fungi of increased tolerance and survival at higher temperatures following slow drying and desiccation. This was less pronounced for *F. pinicola* and *A. chailletii* however it was significant for *P. tremellosa* where four replicates on one isolate (isolate K) even survived 66° C for 30 minutes while all six replicates of the same isolate were killed at 56° C for 60 minutes. Similarly two replicates of a *S. sanguinolentum* isolate survived 56° C for 120 minutes, while in direct heat treatment all replicates were killed by 56/30.

3.3 Heat Treatment of Naturally Infested Wood

Studies using naturally infested wood, both sapwood and heartwood, did not produce useful data as there were not many decay fungi isolated from the control samples. It was then impossible to compare survival rates relevant to different time/temperature combinations and air-drying effects with control wood. It was, however, evident, as in the previous study (Uzunovic and Khadempour 2007), that aggressive, fast growing mold, especially Zygomycetes (*Mucor* spp.), *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp., largely predominated on the substrate and in most cases were the only type of fungi that could be observed or isolated from the wood.

3.4 Test Method

This test has added credence to the Canadian (FPInnovations/CFS) test method for determining thermal death for fungi. All fungi successfully colonized test pieces and there was no contamination in the patch bags during the incubation period. The results following heat treatment were consistent so there was no uncertainty as to whether a fungus survived. In all control replicates, all fungal isolates survived control treatment at 25° C all four tested times. Conversely, all inoculated HT isolates were consistently killed at 61° C or above for 30 minutes and longer test times. All isolates subjected to slow desiccation for 90 days prior to heat treatment (DHT) were killed at 66° C or above for 30 minutes and longer.

During the incubation period we did not always notice visible growth on test wood for some test isolates; there was only visible change in color and no aerial mycelia occurred. Microscopic and culture studies have shown that the fungus was present in the wood and we proceeded with treatments after we established the presence of fungus throughout the wood. We could observe chlamydospores in some isolates; however, as this was not the major focus we did not study formation of chlamydospores in detail. More thorough studies are needed to generate quantifiable data to make a link between the production of chlamydospores and different incubation methods (e.g., slow drying), and to determine whether there is a link or increase in heat tolerance with the chlamydospore formation

4 Discussion

The results obtained here must be considered in the light of the relative pathogenicity of the fungi, the differences between a laboratory test and a commercial heat treatment, and the factors affecting survival of fungi damaged but not killed by the treatment.

The three fungi that survived heat treatment at 56° C for 30 min were *Peniophora* sp, *Phlebia tremellosa* and *Phellinus pini. Peniophora.* sp, and *P. tremellosa* are saprobes of decaying wood and are commonly not listed as pathogens (Allen et al. 1996; Myren 1994). *P. pini* (red ring rot) is a common heart-rot fungus that attacks many species of old large conifers in North America. It enters trees through dead branch stubs and can affect healthy standing trees by producing decay that progresses from the heartwood to the sapwood. It spreads by producing fruiting bodies out of branch stubs on the standing tree and is

unlikely to produce fruiting bodies on lumber. All replicates of this fungus were killed by 61° C at 30 min. *Phellinus chrysoloma*, which also survived 56/30 in previous work but was killed by 61/30 (Uzunovic and Khadempour 2007), is also not listed as a pathogen (Allen et al. 1996; Myren 1994).

The fungi used in our tests and also mentioned in the book "Common tree diseases of British Columbia" (Allen et al. 1996) include *Fomitopsis pinicola* (causing brown crumbly rot), *Stereum sanguinolentum* (causing red heart rot) and *Trichaptum abietinum* (causing pitted sap rot). They all were killed by 56/30. All occur across Canada on a range of hosts including lodgepole pine (Allen et al. 1996). *F. pinicola* is a common wood decay saprophyte that colonizes dead organic material and forest products where it causes a brown heart rot and decay. It may be significant in old-growth forests but is less so in second-growth stands. *S. sanguinolentum* causes heart rot and as a pathogen is more serious in mature pines, spruces and true firs, whereas it is more saprophytic in other wood species. *T. abietinum* affects standing trees where it causes sap and heart rot but can also be found causing decay in unseasoned wood in service. *Sistotrema brinkmannii* can be isolated from decaying wood but has not been shown to cause decay in laboratory tests. It has been found to use chitin as a carbon source and is generally considered to be saprophytic on wood rotting basidiomycetes (Morris 1983). Our tests indicated that all these species are killed by 56/30 or even lower temperature/time combinations.

Known insect associates, *Entomocorticium* sp. and *Amylostereum chailletii* (associated with the *Sirex* wood wasp), were also killed by 56/30 (Kleipzig et al. 1997; Kim et al. 2005; Brett et al. 2007).

Heterobasidion annosum (annosus root rot) is another known pathogen that we did not test but was tested at the Canadian Forest Service, Pacific Forestry Centre. This species often colonizes fresh stumps and can infect and kill healthy adjacent trees through root grafts. It is monitored by some countries and is considered as a plant pathogen of quarantinable importance. It was shown to be killed by 56/30 (Eric Allen, personal communication).

In our tests, fungal tolerance to heat changed as the substrate slowly dried. It has been hypothesized that this is due to development of some spore resting structures (e.g., Chlamydospores) that are induced under harsh conditions. Among the species that are listed as plant pathogens, one isolate out of three of *T. abietinum* survived 56/30 after being air-dried for 90 days (Uzunovic and Khadempour 2007) as well as an isolate of *S. sanguinolentum* in the current test. Both fungi were killed by 56/60 (all isolates) although two replicates of one *S. sanguinolentum* isolate survived 56/120.

While three out of eight of the tested decay fungi found in post-beetle wood can survive the standard heat treatment of 56/30 in a laboratory experiment, this does not necessarily mean that these fungi would survive an industrial 56/30 heat treatment schedule. The method of testing provides a rapid, cool-water-induced drop in temperature once the test temperature/time criteria have been met. In a real-world situation where wood is heated in an industrial kiln, the target internal temperature is frequently exceeded because of a) the heating chamber must achieve 56° C in the coolest part of the chamber to be certified; b) most of the wood cross-section must be heated to a higher temperature for the core to reach 56° C; and c) the speed of heating used to quicken the process normally causes the core temperature to overshoot the target (Smith 1991; Cai 2005; Cai and Garrahan 2006). In the Canadian operating heat treatment manual for heat treatment chambers, each generic schedule contains sufficient safeguarding measures to achieve minimum wood core temperature of 56° C for at least 30 min, and through these measures almost the entire profile of wood is heated at higher temperatures for a longer time (CFIA 2007). Furthermore, cooling is not sudden but often takes a long time because wood is a good insulator. Thus the laboratory test, which merely reaches the target temperature/time criteria, is more conservative in terms of killing pests than the industrial method of probably higher temperature and longer duration than the 56/30 target.

The survival of fungi damaged but not killed by commercial heat treatment would be affected by many factors not included in the laboratory tests with inoculated isolates. The test samples cut from naturally infested substrate represent a real-life situation. The tests using naturally infested samples showed that all

blue-stain and decay fungi were readily killed by 56/30 (Uzunovic and Khadempour 2007) and only a few isolates of common molds survived. Furthermore, in the naturally infested wood that was subjected to slow drying and desiccation, we noticed in both tests significant predominance of aggressive, fast growing, saprotrophic mold fungi (e.g., *Trichoderma,* Zygomycetes, *Penicillium* and *Aspergillus*). They colonized the substrate very quickly precluding successful isolation of blue-stain or decay fungi even from wood that has not been treated. These molds (some genera are commonly used as biocontrol agents e.g., *Trichoderma*) are thus likely to kill or outcompete any surviving pathogenic fungi, preventing their spread from the treated wood under real-life situations.

An additional factor is that most commercial lumber is kiln-dried to below 20% moisture content at the same time as the heat treatment. Fungi do not grow on or out of, nor sporulate on, dry wood, so it is unlikely that there is a pathway for fungi that may be surviving in the wood to spread to living trees.

5 Conclusions

- Five out of eight wood-inhabiting basidiomycetes were eradicated by 56/30 under laboratory conditions.
- Slightly higher temperatures or residence times such as those achieved in practice did kill the three fungi that survived 56/30.
- There was an indication that slow drying of the wood imparted additional heat treatment resistance of some fungi but the significance of this observation remains to be determined.
- Heat treatment reaching 56° C for 30 minutes to the core of wood appears to be an appropriate heat treatment target to significantly reduce pest load and pest transfer through international trade of wood commodities.
- Based on the work completed here and our background knowledge, we believe the chances for the test fungi of potential phytosanitary significance surviving and escaping from post-MPB wood that has been subjected to a standard 56/30 treatment, are remote.

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Table A1. Fungal isolation following heat treatment of test wood inoculated with test isolates (three replicates for 25° C, six for other temperature/ times per isolate).

Temp	Time	Species and different isolates (A-X)																					
		Peniophora sp.			*Entomocorticium* sp.		*Fomitopsis pinicola*			*Phlebia tremellosa*			*Stereum sanguinolentium*			*Amylostereum chailletii*			*Phellinus pini*			*Sistostrema brinkmannii*	
(° C)	(min)	A	B	C	D	E	F	G	H	I	J	K	L	M	N	P	Q	R	S	T	U	W	X
25	<1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	30	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	60	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	120	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
41	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	2	6	6	6	6	6	6	1	5	6	6	6	6	6	6	6	6	6
	60	6	6	6	6	0	6	6	6	6	6	6	0	3	6	6	6	6	6	6	6	6	6
	120	6	6	6	6	0	6	6	6	6	6	6	0	0	6	6	6	6	6	6	6	6	6
46	<1	6	6	6	6	2	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	0	6	6	6	6	6	6	1	5	6	6	6	6	6	6	6	6	6
	60	6	6	6	6	0	6	6	6	6	6	6	0	3	6	6	6	6	6	6	6	6	6
	120	6	6	6	0	0	6	6	6	6	6	6	0	0	6	5	6	6	6	6	6	6	6
51	<1	6	6	6	6	0	6	6	6	6	6	6	0	6	6	6	6	6	6	6	6	0	4
	30	6	6	6	0	0	4	4	3	6	6	6	0	0	6	0	0	6	6	6	6	0	0
	60	6	6	6	0	0	4	0	2	6	6	6	0	0	5	0	0	1	6	6	6	0	0
	120	6	6	6	0	0	0	0	0	6	6	6	0	0	5	0	0	0	6	6	6	0	0
56	<1	6	6	6	0	0	2	0	2	6	6	6	0	0	6	0	0	1	6	6	6	0	0
	30	6	6	6	0	0	0	0	0	6	6	4	0	0	0	0	0	0	6	6	6	0	0
	60	6	6	6	0	0	0	0	0	2	1	0	0	0	0	0	0	0	6	6	6	0	0
	120	0	3	5	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	3	5	0	0
61	<1	5	5	6	0	0	0	0	0	2	2	2	0	0	1	0	0	0	5	5	6	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A2. Survival of six replicates of isolates (three for 25° C) representing four species following HT of inoculated wood after 90 days of prior air-drying.

Temp	Time	*Fomitopsis pinicola**				*Phlebia tremellosa*						*Stereum sanguinolentium*						*Amylostereum chailletii*					
		Isolate G		Isolate H		Isolate I		Isolate J		Isolate K		Isolate L		Isolate M		Isolate N		Isolate P		Isolate Q		Isolate R	
(° C)	(min)	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90	HT	DHT 90
25	<1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	30	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	60	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	120	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
41	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6	6	1	6	5	6	6	6	6	6	6	6	6	6
	60	6	6	6	6	6	6	6	6	6	6	0	6	3	6	6	6	6	6	6	6	6	6
	120	6	6	6	6	6	6	6	6	6	6	0	6	0	6	6	6	6	6	6	6	6	6
46	<1	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	30	6	6	6	6	6	6	6	6	6	6	1	6	5	6	6	6	6	6	6	6	6	6
	60	6	6	6	6	6	6	6	6	6	6	0	6	3	6	6	6	6	6	6	6	6	6
	120	6	6	6	6	6	6	6	6	6	6	0	3	0	5	6	6	5	6	6	6	6	6
51	<1	6	1	6	6	6	6	6	6	6	6	0	5	6	6	6	6	6	6	6	4	6	0
	30	4	0	3	4	6	6	6	6	6	6	0	0	0	0	6	6	0	2	0	0	6	0
	60	0	0	2	5	6	6	6	6	6	6	0	0	0	0	5	6	0	0	0	2	1	0
	120	0	0	0	1	6	6	6	6	6	6	0	0	0	0	5	6	0	0	0	0	0	0
56	<1	0	0	2	4	6	6	6	6	6	6	0	0	0	0	6	6	0	2	0	0	1	0
	30	0	0	0	0	6	6	6	6	4	6	0	0	0	0	0	4	0	0	0	0	0	0
	60	0	0	0	0	2	6	1	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	6	6	6	0	6	0	0	0	0	0	2	0	0	0	0	0	0
61	<1	0	1	0	0	2	6	2	6	2	6	0	0	0	0	1	1	0	0	0	0	0	0
	30	0	2	0	0	0	0	0	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	4	0	5	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
66	<1	0	0	0	0	0	0	0	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
71	<1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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